

CLAIMS

What is claimed is:

1. A method for quantifying an initial ratio of the amounts of at least two nucleic acids of interest in a sample by means of a multiplex nucleic acid amplification reaction, comprising:
 - amplifying the nucleic acids of interest in the amplification reaction;
 - measuring the amount of at least two nucleic acids of interest at at least two different time points in the reaction;
 - determining from at least two of the measurements the amplification rate of the at least two nucleic acids of interest;
 - comparing the rates with a reference; and
 - determining, from the comparison, the initial ratio of the amounts of the at least two nucleic acids of interest in the sample.
2. The method according to claim 1, wherein at least one variable factor in the nucleic acid amplification reaction is adjusted in order to allow detectable levels of all nucleic acids of interest to be reached before an amplification and/or detection limit of one or more of the nucleic acids of interest is reached.
3. The method according to claim 2, wherein the variable factor affects an amplification efficiency of one nucleic acid of interest to a different extent as compared to another nucleic acid of interest.
4. The method according to claim 2 or claim 3, wherein the variable factor comprises the concentration of at least one primer.

5. The method according to claim 2, claim 3, or claim 4, wherein the concentration of at least one primer is significantly different from the concentration of at least one other primer.

6. The method according to claim 2, claim 3, claim 4 or claim 5, wherein the variable factor comprises the concentration of at least one set of primers capable of annealing to a nucleic acid of interest.

7. The method according to claim 2, claim 3, claim 4, claim 5, or claim 6, wherein the concentration of at least one set of primers capable of annealing to a nucleic acid of interest is significantly different from the concentration of at least one other set of primers capable of annealing to a nucleic acid of interest.

8. The method according to claim 2, claim 3, claim 4, claim 5, claim 6, or claim 7, wherein the variable factor comprises the concentration of salt.

9. The method according to claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, or claim 8, wherein the nucleic acids of interest comprise independent nucleic acids of interest.

10. The method according to claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, or claim 9, wherein at least one of the nucleic acids of interest comprises RNA.

11. The method according to claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, claim 9, or claim 10, wherein at least one of the nucleic acids of interest comprises DNA.

12. The method according to claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, or claim 11, wherein the multiplex amplification reaction comprises NASBA.

13. A method for determining functioning of a cellular organism, said method comprising:

determining the ratio of the amount of a first nucleic acid in relation to the amount of a second nucleic acid in a sample obtained from the cellular organism,

wherein the ratio is quantified with the method according to claim 1, claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, claim 11, or claim 12.

14. The method according to claim 1, claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, claim 11, claim 12, or claim 13, wherein at least one of the nucleic acids of interest comprises an endosymbiont cellular organelle nucleic acid.

15. The method according to claim 14 wherein the ratio comprises the ratio of the amount of an endosymbiont cellular organelle nucleic acid in relation to the amount of a nuclear nucleic acid in the sample.

16. A method for determining the staging of a disease, said method comprising:

determining the ratio of the amount of a first nucleic acid in a sample obtained from an organism suffering from, or at risk of suffering from, the disease in relation to the amount of a second nucleic acid,

wherein the ratio is quantified with the method according to claim 1, claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, claim 11, or claim 12.

17. The method according to claim 16, wherein the disease is selected from the group consisting of an HIV-related disease, a tumor-related disease, an angiogenic process, and a combination of any thereof.

18. The method according to claim 16 or claim 17, wherein the first cellular organelle nucleic acid and the second nucleic acid comprise DNA and RNA.

19. A method for determining therapeutic activity and/or possible side effects of a compound, said method comprising:

determining the ratio of the amount of a first nucleic acid in a sample obtained from an organism in relation to the amount of a second nucleic acid with the method according to claim 1, claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, claim 11, or claim 12.

20. The method according to claim 19, wherein the therapeutic activity comprises a therapeutic activity against an HIV-related disease, a tumor-related disease, or both an HIV-related disease and a tumor-related disease.

21. The method according to claim 19, wherein the compound comprises a nucleoside and/or nucleotide analogue.

22. The method according to claim 20, wherein the compound comprises a nucleoside and/or nucleotide analogue.

23. The method according to claim 21 or claim 22, wherein the nucleoside and/or nucleotide analogue comprises fludarabine, mercaptopurine, tioguanine, cytarabine, fluorouracil, and/or gemcytabine.

24. The method according to claim 19, claim 20, claim 21, claim 22, or claim 23, wherein the compound comprises AZT, ddI, ddC, d4T, 3TC and/or tenofovir, and/or abacavir.

25. The method according to claim 19, wherein the therapeutic activity comprises a therapeutic activity against an angiogenic process.

26. The method according to claim 25, wherein the compound or medicament comprises at least one of the following drugs: 2ME2, angiostatin, angiozyme, Anti-VEGF RhuMAb, apra (CT-2584), avicine, benefin, BMS275291, carboxyamidotriazole, CC4047, CC5013, CC7085, CDC801, CGP-41251 (PKC 412), CM101, combretastatin A-4 prodrug, EMD 121974, endostatin, flavopiridol, Genistein (GCP), green tea extract, IM-862, ImmTher, interferon alpha, interleukin-12, Iressa (ZD1839), marimastat, metastat (Col-3), Neovastat, octreotide, paclitaxel, penicillamine, Photofrin, Photopoint, PI-88, Prinomastat (AG-3340), PTK787 (ZK22584), RO317453, Solimastat, Squalamine, SU 101, SU 5416, SU-6668, Suradista (FCE 26644), Suramin (Metaret), tetrathiomolybdate, thalidomide, TNP-470, and Vitaxin.

27. A method for determining toxic activity of a candidate compound for causing malfunctioning of a cellular organism, said method comprising:

determining a ratio of the amount of a first nucleic acid in a sample obtained from an organism in relation to an amount of a second nucleic acid with a method according to claim 1, claim 2, claim 3, claim 4, claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, claim 11, or claim 12.

28. The method according to claim 19, claim 20, claim 21, claim 22, claim 23, claim 24, claim 25, claim 26, or claim 27 wherein the organism or an essentially related organism has been provided with the compound.

29. A method for determining selective activity of a candidate compound against a first organism, said method comprising:

determining therapeutic activity and/or possible side effects of the candidate compound with the method according to claim 19 or claim 20 and/or determining toxic activity with the method according to claim 27.

30. The method according to claim 29 further comprising providing an essentially unrelated second organism with the compound.

31. The method according to claim 30 wherein the first organism comprises a pathogen and the second organisms comprises a host for the pathogen.

32. The method according to any one of claims 13-31, wherein the first nucleic acid comprises an endosymbiont cellular organelle nucleic acid.

33. The method according to any one of claims 13-32, wherein the second nucleic acid comprises a nuclear nucleic acid.

34. The method according to any one of claims 13-33, wherein the first nucleic acid and/or the second nucleic acid is obtained from a peripheral blood mononuclear cell and/or a fibroblast.

35. A diagnostic kit comprising:

at least one means for performing the method according to any one of claims 1-34, and suitable packaging.

36. The diagnostic kit of claim 35, comprising at least one primer or probe selective for amplification and/or detection of a nucleic acid related to or derived from endosymbiont cellular organelles.

37. The diagnostic kit of claim 35 or claim 36, comprising a significantly different amount of at least one primer as compared to the amount of at least one other primer.

38. The diagnostic kit of claim 35, claim 36, or claim 37, comprising a significantly different amount of at least one set of primers capable of annealing to a nucleic acid of interest as compared to the amount of at least one other set of primers capable of annealing to a nucleic acid of interest.

39. The diagnostic kit of claim 36, claim 37, or claim 38, wherein the at least one primer or probe is listed in Table 1.

40. Use of a compound obtainable or selectable by the method according to any one of claims 19-34 in the preparation of a medicament, a herbicide, an insecticide, an anti-parasiticum, an cytostatic agent or cytotoxic agent.

41. A medicament, a herbicide, an insecticide, an anti-parasiticum, an cytostatic agent or cytotoxic agent obtainable or selectable by the method according to any one of claims 19-34.